Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 14-32 are pending in the application, with claims 14 and 32 being the independent claim. Claims 1-13 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. New claims 14-32 are sought to be added to more particularly define and claim the present invention. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

I. Support for New Claims

Support for new claims 14-31 can be found throughout the specification, for example, at page 3, lines 18-21, page 7, lines 8-11, page 13, lines 10-18, page 14, lines 6-16, page 16, lines 16-21, page 40, lines 15-27, as well as original claims 1-11.

II. Rejections under 35 U.S.C. § 112, First Paragraph: Written Description

The Examiner newly rejects claims 1-2 and 5-13 under 35 USC § 112, first paragraph, because, according to the Examiner, these claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled

in the art that the inventors, at the time the application was filed, had possession of the claimed invention. *See,* Paper No. 10, page 4. According to the Examiner,

> The instant claims encompass the utilization of a nucleotide molecule encoding FPGS derived from any species (both mammalian and non-mammalian species). However, apart from the exemplification showing a human FPGS cDNA gene product that is capable of polyglutamating antifolate drugs. . .to enhance their cytotoxicity or to increase their therapeutic efficacy, the instant specification fails to teach a representative number of species of nucleotide encoding FPGS derived from any organisms that are capable of [mediating] the same desired anti-neoplastic cell effects as encompassed within a broad scope of the instant claims. At the effective filing date of the present application, mouse and human FPGS genes are the only two mammalian FPGS genes that have been cloned and sequenced along with the bacterial FPGS genes from *E. coli* and *Lactobacillus casei.*

Id. at page 5. The Examiner concludes that the skilled artisan could not envision the detailed structure of any nucleotide molecule encoding FPGS apart from those mentioned above. Applicants respectfully traverse the rejection.

The specification, at page 13, lines 25-28, states that: "The FPGS gene may be from any species. FPGS cDNA from many species are known to those skilled in the art , and their nucleotide sequences can be obtained from the GenBank Sequence Database. The mammalian FPGS gene is preferred. The human FPGS gene is particularly preferred."

It is well established law that a specification need not teach, and preferably omits, information that is well-known to those of ordinary skill in the art. *See Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384 (Fed. Cir. 1986); *Lindemann Maschinenfabrik v. American Hoist and Derrick*, 730 F.2d 1452, 1463 (Fed. Cir. 1984). One of ordinary skill in the art is also deemed to know not only what is considered well-known, but also where to search for any needed starting materials. *See In re Howarth*, 210

USPQ 689, 692 (CCPA 1981). As detailed in the present specification, DNA sequences encoding FPGS from several sources (mammalian and non-mammalian) were well-known in the art at the time of filing of the present invention.

Significantly, Applicants respectfully disagree with the Examiner's statement that "At the effective filing date of the present application, mouse and human FPGS genes are the *only two* mammalian FPGS genes that have been cloned and sequenced along with the bacterial FPGS genes from *E. coli* and *Lactobacillus casei*." (Emphasis added).

The Table below presents GenBank accession numbers and/or reference citations, showing that at the time of filing, FPGS was sequenced from other species, beyond the four mentioned by the Examiner. This information would have been readily available to those skilled in the art, especially given Applicants guidance in the specification to consult the GenBank sequence database for other FPGS sequences.

Species	GenBank Release Date	Journal Reference(s); GenBank Accession No.
Mammalian		
Homo sapiens	12-JUN-1993	*Proc. Natl. Acad. Sci. U.S.A.* 89 (19), 9151-9155 (1992); M98045
Mus musculus	28-SEP-1996	*J. Biol. Chem.* 270 (45), 26918-26922 (1995); *J. Biol. Chem.* 271 (39), 23820-23827 (1996); U33557
Cricetulus griseus	27-JAN-1996	*J. Biol. Chem.* 270 (16), 9579-9584 (1995); U14938
Rattus sp.	08-OCT-1998	AI176002.1
Non-Mammalian		
Lactobacillus casei	14-FEB-1996	*J. Biol. Chem.* 265 (5), 2492-2499 (1990); J05221
Treponema pallidum	16-JUL-1998	*Science* 281 (5375), 375-388 (1998); AAC65325.1
Streptomyces coelicolor A3(2)	03-MAR-1998	*FEMS Microbiol. Lett.* 159 (2), 283-291 (1998); Y13070
Neisseria gonorrhoeae	N/A	*Mol. Gen. Genet.* 250(3), 277-85 (1996)
Schizosaccharomyces pombe	13-MAR-1998	*DNA Res.* 4 (6), 363-369 (1997); D89257
Aquifex aeolicus	25-MAR-1998	*Nature* 392 (6674), 353-358 (1998); AAC07789.1
Rickettsia prowazekii	11-NOV-1998	*Nature* 396 (6707), 133-140 (1998); CAA14985.1
Thermotoga maritima	02-JUN-1999	*Nature* 399 (6734), 323-329 (1999); AAD35259.1
Helicobacter pylori 26695	06-APR-1999	*Nature* 388 (6642), 539-547 (1997); AAD08584.1
Haemophilus influenzae Rd	10-MAY-1999	*Science* 269 (5223), 496-512 (1995); AAC22914.1
Escherichia coli	26-APR-1993	*J. Biol. Chem.* 262 (25), 12337-12343 (1987); M32445
Bacillus subtilis	26-APR-1993	*J. Bacteriol.* 171 (11), 6043-6051 (1989); AAB59021
Neurospora crassa	28-JUN-1997	*Phytochem* 49(8), 2221-32 (1998); AF005040
Candida albicans	22-JUN-1999	AF156928

Accordingly, the rejection under 35 USC § 112, first paragraph, for insufficient written description should be reconsidered and withdrawn.

III. Rejections under 35 U.S.C. § 112, First Paragraph: Enablement

The Examiner rejected claims 1-3 and 5-13 under 35 USC § 112, first paragraph, because, according to the Examiner, the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. *See* Paper No. 10, page 8.

The Examiner provides four separate grounds for rejecting Applicants' claims under 35 USC § 112, first paragraph, based on lack of enablement. Applicants address each of these grounds in turn below.

A. The FPGS Nucleotide Sequence

The Examiner contends that there is a lack of guidance in the specification regarding the availability of a nucleotide sequence encoding FPGS, other than human, mouse, *E. coli* or *L. casei* FPGS.

As discussed above regarding the written description rejection, Applicants submit that there are additional sequenced FPGS genes from species other than human, mouse, *E. coli* or *L. casei* that would have been known to those skilled in the art from the GenBank sequence database (and/or art) at the time of filing. Accordingly, it would require no more than routine experimentation for one skilled in the art to practice the claimed method with

any FPGS nucleotide sequence whose nucleotide sequence was known. Reconsideration and withdrawal of this rejection are respectfully requested.

B. In Vivo Delivery of a Genetic Vector

The Examiner asserted that the full scope of the claims is not enabled because the "claims encompass any route of delivering a vector of gene delivery comprising a nucleotide molecule encoding FPGS into neoplastic cells *in vivo*," and that "vector targeting *in vivo* to desired cells or tissues, for this instance neoplastic cells, continues to be unpredictable and inefficient." *See* Paper No. 10, page 10. In support of this position, the Examiner cited four references that are intended to illustrate the technical difficulties associated with delivering genetic vectors to target cells *in vivo* by methods other than direct intratumoral injection (Dang, C.V., *et al.*, *Clin. Cancer Res. 5*:471-474 (1999); Miller, N. and Vile, R., *FASEB J. 9*:190-199 (1995); Deonarian, M.P., *Exp. Opin. Ther. Patents 8*:53-69 (1998); and Verma, I.M. and Somia, N, *Nature 389*:239-242 (1997)).

Applicants assert that the references cited by the Examiner merely set forth technical hurdles that need to be overcome in order to *increase the efficiency* with which genetic vectors are targeted to cells *in vivo*. They do not, however, indicate that genetic vector delivery to target cells is impossible or infeasible. Deonarian, in fact, describes experimental results in which genetic vectors were successfully delivered to liver cells *in vivo* using ligand-targeted receptor-mediated endocytosis of polyplexes:

> Reporter gene delivery experiments *in vivo* showed 85% of the injected DNA was taken up by the liver by 10 min. A great deal of research has followed, including *in vivo* gene delivery of albumin to rats with LDL receptor deficiency. An average of 1000 copies of the plasmid were found per

> hepatocyte resulting in a level of 34 μg/ml human albumin in the serum of animals 2 - 4 weeks after injection and partial hepatectomy.

Deonarian at 59, left column. At page 15 of Paper No. 10, the Examiner contends that Deonarain does not teach that the injected DNA can be delivered efficiently to neoplastic cells other than the liver, and that the claims are not drawn to killing neoplastic cells in a liver.

First, Applicants' claims do not exclude liver cancer cells. Second, Applicants are merely citing Deonarain as an examplary publication where gene delivery was successful without requiring direct delivery.

The Dang reference concludes with the following sentence: "Whereas setbacks in gene therapy were clearly recognized and discussed, there was a unique level of enthusiasm that many of these obstacles could be overcome with meticulously designed basic and clinical studies." *See* Dang at page 474, right column. Therefore, the references cited by the Examiner actually support, rather than refute, Applicants' contention that the specification enables the full scope of the claims.

The Examiner has grounded the enablement rejection on the alleged low efficiency with which genetic vectors can be delivered *in vivo*; the Examiner does not contend that *in vivo* gene delivery is generally impractical. For example, the Examiner stated that

> [t]he instant specification fails to teach one of skilled [sic] in the art how to overcome the unpredictability for *in vivo* vector targeting, such that an *efficient transfer and expression* of a FPGS gene could be achieved in neoplastic cells of solid or non-solid tumors through any and all routes of delivery such that upon treatment with a chemotherapeutic agent, the agent is activated by the FPGS gene product to effect the killing of said neoplastic cells.

Paper No. 10, pages 11-12 (emphasis added).

Applicants note that to satisfy the enablement requirement of 35 U.S.C. § 112, first paragraph, a specification need only enable the "full scope of the claimed invention." *In re Wright*, 999 F. 2d 1557,1561 (Fed. Cir. 1993). Furthermore, "as concerns the breadth of a claim relevant to enablement, the only concern should be whether the scope of enablement provided to one skilled in the art by the disclosure is commensurate with the scope of protection sought by the claims." MPEP § 2164.08.

The claimed methods are directed to "A method of enhancing the cytotoxic sensitivity of neoplastic cells to an antifolate drug" by "(a) delivering into said neoplastic cells a vector, said vector comprising a nucleotide molecule encoding folylpolyglutamyl synthetase (FPGS), operably linked to a promoter, wherein said FPGS is expressed in said neoplastic cells at a level higher than the endogenous FPGS level of said neoplastic cells; (b) treating said neoplastic cells with an antifolate drug that is polyglutamated by said FPGS; and (c)enhancing the cytotoxic sensitivity of said neoplastic cell to said antifolate drug.

There is nothing in Applicants' claims requiring that the vector carrying the FPGS gene be delivered into neoplastic cells with any particular minimum level of efficiency, so long as the cytotoxic sensitivity of the neoplastic cells are enhanced following antifolate treatment.

The above notwithstanding, it was recognized in the art at the time of Applicants' application, that a major advantage associated with gene delivery systems utilizing so called "suicide genes," such as FPGS, is that only a low level of gene delivery is required to exert anti-neoplastic effects.

> Suicide genes are used in a significant number of the cancer trials because they have a number of advantages. (1) Suicide genes and their prodrug are toxic to chemotherapy resistant tumors. (2) Only short-term gene expression is required. *(3) Only a fraction of the tumor cells within the tumor mass (>10%) needs to express the suicide gene to kill the entire tumor.* (4) Gene-modified tumor cells that die after exposure to the prodrug stimulate an immune response, which in some cases has been shown to be stronger than that to irradiated tumor cells. Taken together, these characteristics allow for cytoreduction of the tumor mass through "molecular surgery" by killing the tumor cells that have been genetically modified with the suicide gene.

Freeman, S.M., *et al.*, *Semin. Oncology 23*:31-45 (1996) (emphasis added, Table references omitted; cited and incorporated by reference in the specification at page 4, line 10, and listed in the Information Disclosure Statement filed on November 2, 2000); *See also* Verma and Somia at 239 ("In the brain, however, gene transfer to *just a few hundred cells* could considerably benefit patients with neurological disease," emphasis added).

Finally, in this regard, the Applicants have provided evidence for a positive "bystander effect" in culture and *in vivo* using the presently claimed method. *See*, specification, pages 37-40. It is well-known in the gene therapy art that evidence of a positive bystander effect is important because of low transduction efficiencies of a particular gene. The "bystander effect" refers to the ability of transduced tumor cells to cause cytotoxicity in neighboring non-transduced tumor cells. It is well known that this is an important prerequisite for success under real-world conditions, where the transfer of genes to cells within a tumor is substantially less than 100%.

Thus, as recognized by those skilled in the art, the successful application of the invention does not necessarily require high levels of efficiency of genetic vector delivery.

Moreover, there are several examples in the literature at the time the present application was filed that demonstrate the effectiveness of *in vivo* genetic vector delivery by methods other than direct inoculation of tumors. For instance, Deonarian, discussed above, describes successful results involving the *in vivo* delivery of genetic vectors to liver cells. As another example, Lan *et al.*, *Cancer Res. 57*:4279-4284 (1997), demonstrated the delivery of an i.p.-administered adenoviral vector to gastric carcinoma cells. (Lan was cited and incorporated by reference in the specification at page 21, line 19, and was listed in the Information Disclosure Statement filed on November 2, 2000). In addition, Nakanishi, *Crit. Rev. Therapeu. Drug Carrier Systems 12*:263-310 (1995), provides an overview of systems for gene transfer into tissue cells and summarizes several studies which illustrate the *in vivo* delivery of retroviral, adenoviral, adeno-associated viral, and herpesviral vectors. Nakanishi further summarizes the state of *in vivo* gene transfer as of 1995:

> *In vivo* gene transfer is an approach to transfect tissue cells *in situ* by introducing gene transfer vectors through direct injection, through perfusion with catheters, or through an intravenous injection. This approach is more practical than *ex vivo* gene transfer and will become the major route for therapeutic gene transfer in the future. *In vivo* transfer may be applied to a wide variety of tissues and cells, and many vector systems other than retroviral vectors have been reported to be adopted for *in vivo* transfection.

Nakanishi at page 267 (Nakanishi was cited and incorporated by reference in the specification at page 16, lines 10-11, and was listed in the Information Disclosure Statement filed on November 2, 2000). Finally, Roth *et al.*, relied upon by the Examiner to support an obviousness rejection under 35 U.S.C. § 103, show, according to the Examiner, that certain gene-based therapies for cancer, including drug sensitization with genes for prodrug

delivery similar in nature with the presently claimed invention, have resulted in tumor regressions or killing cancer cells (see the entire article, particularly pages 21-24).

Thus, the state of the art as of the effective filing date of the present application clearly indicates that the *in vivo* delivery of genetic vectors to target cells by methods besides direct inoculation is feasible and has important clinical applications in the field of gene therapy.

Another issue raised by the Examiner concerns host immunological responses to the delivered vectors, as a factor limiting an effective gene therapy. As discussed in the specification at page 42, lines 2-13, one of the advantages of FPGS gene therapy is that the gene product expressed by the tumor cells (FPGS) is not a foreign enzyme, and therefore should not elicit an immune response, as might be the case with expression of a foreign "suicide" gene (*i.e.*, HSV-thymidine kinase, bacterial cytosine deaminase), which may generate an immune response before the tumor cells are able to mediate an effective bystander effect.

To summarize, the references cited by the Examiner in support of the rejection merely indicate that, in certain contexts, technical difficulties may exist that impede the delivery of genetic vectors with optimum efficiency. Applicants' claims, however, do not specify any minimum level of genetic vector transfer efficiency. The Examiner has not established that the *in vivo* delivery of genetic vectors by methods other than direct injection is of such low efficiency so as to be regarded as impracticable. In fact, at the time the present application was filed, the scientific literature (including even the references cited by the Examiner) was replete with examples of successful *in vivo* genetic vector delivery using methods other than direct injection; that is, it would not require undue experimentation for

a skilled artisan to practice the full scope of Applicants' invention, including the delivery of genetic vectors by *in vivo* methods. The Examiner has not met his burden in establishing a *prima facie* case of non-enablement. Therefore, in addition to *in vitro* genetic vector delivery and the delivery of genetic vectors by direct inoculation, Applicants' claims are fully enabled with respect to *in vivo* genetic vector delivery methods.

C. Replication Competent Viral Vectors

The Examiner also asserted that the present claims "encompass the use of replication competent viral vectors," but that "[n]either the instant specification nor the prior art at the effective filing date of the present application teaches the use of replication competent viral vectors such as retrovirus, adenovirus or lentivirus (HIV-1 and HIV-2) for achieving therapeutic results via gene therapy." *See* Paper No. 10, page 12. The Examiner further suggests that the use of replication competent viral vectors would impede the practice of the claimed methods:

> It is unclear whether the treated individual having neoplastic cells succumbs to the cytotoxic effects of replication competent viral vectors prior to any therapeutic effects contemplated by Applicants could be attained. Furthermore, neoplastic cells infected with replication competent viruses can be lysed or killed by replication competent recombinant viruses prior to any effective accumulation of polyglutamated antifolates could be attained in the infected neoplastic cells to mediate the killing of neoplastic cells as contemplated by Applicants.

Paper No. 10, page 12.

In essence, the Examiner has based the enablement rejection on the presence of alleged inoperative embodiments, *i.e.*, methods for killing neoplastic cells using replication

competent viral vectors. As an initial matter, Applicants submit that the Examiner has not provided any objective evidence in support of his apparent conclusion that the use of replication competent viral vectors would interfere with the practice of the claimed methods. Therefore, the Examiner has not satisfied his burden in establishing a *prima facie* case of non-enablement. *See In re Wright,* 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993).

Moreover, Applicants note that the presence of inoperative embodiments within the scope of a claim does not necessarily render a claim non-enabled.[1] *See Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984). The standard is whether a skilled artisan could determine which embodiments of the claimed invention would be inoperative or operative with expenditure of no more effort than is normally required in the art. *See id.*, *see also In re Angstadt*, 537 F.2d 498, 502-503, 190 USPQ 214, 218 (CCPA 1976).

Further, the Examiner contends that neither the specification nor the art teach the use of replication competent viral vectors. Applicants respectfully disagree. Page 19, lines 13-21, of the specification states:

> Preferably, the viral genomes of the viral vectors used in the invention should be modified to remove or limit their ability to replicate, however, *replication conditional viruses will also be useful in the present invention, as will replicating vectors that are capable of targeting certain cells. See, e.g.*, Zhang, J., *et al., Cancer Metastasis Rev. 15*:385-401 (1996). Chase, M., *et al. (Nature Biotechnol. 16*:444-448 (1998))

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[1]As noted by one commentator, "nearly all claims can be read on inoperative embodiments if one is deliberately setting out to sabotage the invention." Janicke, "Patent Disclosure -- Some Problems and Current Developments (Part II)," 52 J. Pat. Off. Soc'y 757, 772-773 (1970).

> used a herpes virus with an inactivated viral ribonucleotide reductase gene that selectively delivered P450 2B1 to tumor cells that overexpress the mammalian ribonucleotide reductase enzyme, which is required for this modified virus to replicate. (Emphasis added).

Thus, contrary to the Examiner's assertion, the present specification does indeed provide guidance for using a viral vector that is not replication-*defective*. Further, Applicants direct the Examiner's attention to the following U.S. Patents: 5, 585,096 (1996) and 6,106,826 (2000), which are directed to viral vectors that are replication competent, yet avirulent, that find use in mammalian gene therapy.

Applicants assert that it would take no more than minimal effort on the part of the skilled artisan to ascertain which viral vectors would and would not be appropriate for use within the scope of the claimed invention. The state of the art as of the effective filing date of the present application was such that the various classes and variants of viral vectors (and other vehicles for the delivery of genetic material to cells) were well defined and understood. *See, e.g.,* Nakanishi, *supra*, for an overview of various methods for gene transfer into cells.

Applicants submit that it is unnecessary for Applicants to expressly exclude from the scope of the present claims the use of replication competent vectors, as several types of these vectors (replicating vectors that are capable of targeting certain cell types or replication conditional vectors, for example) find use in the practice of the claimed methods.

D. Mammalian Artificial Chromosomes

The Examiner asserted that claim 11 specifically encompasses the use of mammalian artificial chromosomes as a non-viral gene delivery of FPGS gene for killing neoplastic cells

but that "the instant specification fails to provide any specific teachings regarding to [sic] the making or using of any mammalian artificial chromosome for killing a neoplastic cell in a method as claimed." *See* Paper No. 10, page 13.

Applicants note that they are not limited to the confines of the specification to provide the necessary information to enable the invention. *See In re Howarth*, 654 F.2d 103, 105-6, 210 USPQ 689, 692 (CCPA 1981). Furthermore, an Applicant need not supply information that is well known in the art. *Id.*, 654 F.2d at 105-6, 210 USPQ at 692; *see also In re Brebner*, 455 F.2d 1402, 173 USPQ 169 (CCPA 1972) (finding a disclosure enabling because the procedure for making the starting material, although not disclosed, would have been known to one of ordinary skill in the art as evidenced by a Canadian patent). "'That which is common and well known is as if it were written out in the patent and delineated in the drawings.'" *Howarth*, 654 F.2d at 106, 210 USPQ at 692 (quoting *Webster Loom Co. v. Higgins et al.*, 105 U.S. (15 Otto.) 580, 586 (1881)). In addition, one of ordinary skill in the art is deemed to know not only what is considered well known in the art, but also where to search for any needed starting materials. *Id.*

Applicants assert that the construction and use of mammalian artificial chromosomes was well known in the art as of the effective filing date of the present application. *See e.g.*, Specification at page 20, line 9-10 (citing Ascenzioni, *et al., Cancer Lett. 118*:135-142 (1997), which provides an overview of the technology regarding mammalian artificial chromosomes). Thus, it would have been unnecessary, indeed improper, for Applicants to provide detailed instructions regarding the construction of mammalian artificial chromosomes.

The Examiner cites a passage from Calos, *Trends Genet. 12*:463-466 (1996), that allegedly lends support to the conclusion that "it would have required undue experimentation for a skilled artisan to make and use this particular embodiment of the presently claimed invention." *See* Paper No. 10, page 13. The passage cited from the Examiner is as follows:

> A vector of this size is far beyond the size of vectors in current use for gene therapy and poses problems of major dimensions, particularly for the manufacture and delivery of vector DNA. Therefore, while construction of artificial chromosome vectors has not yet been realized, once it is, a series of challenging technical barriers will have to be surmounted before such molecules could reasonably be used as gene therapy vectors.

Calos at page 464, right column.

Applicants note that, in the above-quoted passage, the author is referring to artificial chromosomes created by a very specific strategy, namely, the progressive reduction of existing chromosomes using telomere-mediated fragmentation. *See id.* Moreover, when read in its full context, the author's statement amounts to, at most, an educated guess as to the minimum size that might be achieved for an artificial chromosome based on the *prediction* that "[t]he final size [of an artificial chromosome produced by telomere-mediated fragmentation] will *probably* be driven primarily by the minimal size of a stable centromere, *which appears* to be about 1 Mb." *See id,* (emphasis added).

Therefore, the only conclusion that can be reasonably drawn from the statements in Calos is that technical difficulties *may* be encountered in the production of artificial chromosomes when they are generated by the progressive reduction of existing chromosomes using telomere-mediated fragmentation, *assuming* that the minimal size of a

stable centromere, is about 1 Mb. Applicants maintain that such tentative conclusions do not suggest that the practice of the claimed invention insofar as it encompasses the use of mammalian artificial chromosomes would require any more than routine experimentation.

Applicants direct the Examiner's attention to the attached two Abstracts: (1) Huxley, C., "Mammalian Artificial Chromosomes; a New Tool for Gene Therapy," *Gene Therapy 1*:7-12 (1994); and (2) Vos, JM, "Mammalian Artificial Chromosomes as Tools for Gene Therapy", *Curr Opin Genet Dev 8*:351-9 (1998), which were also available to those skilled in the art as of Applicants' filing date, and provide guidance on the use of Mammalian Artificial Chromosomes in the context of gene therapy.

Even if the Examiner's assessment of mammalian artificial chromosomes was correct, however, (a proposition which Applicants explicitly traverse), the rejection would amount to no more than an rejection on the basis of an alleged inoperative embodiment[2]. As stated above, the presence of inoperative embodiments within the scope of a claim does not necessarily render a claim non-enabled. *See Atlas Powder Co.*, 750 F.2d at 1577, 224 USPQ at 414. A rejection for lack of enablement on the basis of an inoperative embodiment is only proper when it is shown that a skilled artisan would require more than routine experimentation to distinguish the inoperative from the operative embodiments. *See id.*

With respect to the present claims, the Examiner has provided no evidence or arguments to suggest that the reasonable practice of the claimed invention would be

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[2]Applicants note that the only claim that explicitly defines the vector for gene delivery as a mammalian artificial chromosome is claim 11 wherein a mammalian artificial chromosome is included within a Markush group of vectors for gene delivery. In the remainder of the claims, the use of mammalian artificial chromosomes is merely an alleged inoperative embodiment.

substantially impeded due to the presence of mammalian artificial chromosomes within the scope of the claims. Nor has the Examiner set forth any indication that it would require undue experimentation to distinguish the operative from the supposed inoperative embodiments of the claimed invention. Accordingly, the presence of mammalian artificial chromosomes within the scope of the present claims does not provide a permissible basis of rejection under 35 USC § 112, first paragraph.

E. Summary

Applicants assert that the Examiner, in providing the four grounds of rejection discussed above, has not met his initial burden of establishing a reasonable basis to question the enablement provided for the claimed invention. *See In re Wright*, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). Accordingly, Applicants respectfully request that the rejection under 35 USC § 112, first paragraph, for lack of enablement, be reconsidered and withdrawn.

IV. Rejections under 35 USC § 112, Second Paragraph

The Examiner rejected claims 1-3 and 5-11 under 35 USC § 112, second paragraph, because, according to the Examiner, these claims are indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. *See* Paper No. 10, page 18.

A. Claim 1

The Examiner asserted that in amended claim 1, and in claims dependent thereon, the phrase "said nucleotide molecule directs the production of said FPGS" in step (a) is unclear. *See* Paper No. 10, page 18.

Applicants believe that the newly submitted claims do not contain the allegedly objectionable language and also recite, for clarity, that the nucleotide molecule encoding FPGS is operably linked to a promoter.

Thus, this rejection under 35 USC § 112, second paragraph, has been overcome and should, accordingly, be withdrawn.

B. Claim 6

The Examiner asserted that, in claim 6, the phrases "said FPGS gene" and "said chemotherapeutic agent" were lacking antecedent basis in claim 1. See Paper No. 10, page 19.

This rejection is rendered moot by the cancellation of claim 6. This rejection under 35 USC § 112, second paragraph, should be withdrawn.

V. Rejections under 35 U.S.C. § 103

A. The Examiner rejects claims 1-3, 5-6 and 10-12 under 35 USC § 103(a), because, according to the Examiner, these claims are unpatentable over Kim *et al.*, *J. Biol. Chem. 268*:21680-21685 (1993) ("Kim") as evidenced by Osborne *et al.*, *J. Biol. Chem. 268*:21657-21664 (1993) ("Osborne"), and in view of Garrow *et al.*, *Proc. Nat'l. Acad. Sci.*

89:9151-9155 (1992) ("Garrow") and Roy *et al.*, *J. Biol. Chem. 272*: 6903-6908 (1997) ("Roy"). *See* Paper No. 10, pages 19-20.

This rejection is apparently directed to the *in vitro* aspect of the claimed method.

Kim teaches that mutant CHO cells, lacking FPGS activity, exhibit increased sensitivity to pulses of MTX in cell culture after being transfected with an FPGS expression cassette. Thus, Kim teaches that FPGS cDNA transfection restored cytotoxic sensitivity of FPGS-deficient CHO cells to methotrexate. As acknowledged by the Examiner, Kim does not teach the transformation or transfection of neoplastic cells. In addition, Kim does not teach the transformation or transfection of cells which have some endogenous FPGS activity (like neoplastic cells), nor show that such transformation or transfection with an FPGS gene can enhance the neoplastic cell's cytotoxic sensitivity to an anti-folate drug. Osborne does not correct these fundamental deficiencies.

Garrow teaches the cloning of a human FPGS. Garrow teaches that transfecting the cloned FPGS into mutant CHO cells lacking FPGS activity restored the ability of the transfected mutant cells to grow in culture in the absence of purines and thymidine. Garrow does not teach the delivery of a vector comprising a nucleotide molecule that encodes an FPGS into *neoplastic cells*, nor does Garrow teach the treatment of cells expressing FPGS with an antifolate drug. Further, Garrow doesn't teach or suggest that transformation or transfection of neoplastic cells with an FPGS gene can enhance the neoplastic cell's cytotoxic sensitivity to an anti-folate drug.

Roy teaches that L1210 cell variants which express either decreased or increased levels of FPGS (compared with parental lines) display corresponding differences in resistance to folate analogues. According to the Examiner, Roy teaches that L1210 tumor

cells resistant to MTX have a decrease in the rate of FPGS mRNA transcript formation, resulting in lower FPGS activity (page 6907, col.2, first full paragraph).

The Examiner provided the following justification for the rejection:

> It would have been obvious and within the skills of an ordinary skilled artisan to modify the method disclosed by Kim by transforming a non-viral vector (or a prokaryotic vector) comprising a DNA sequence encoding human FPGS into neoplastic or tumor cells resistant to methotrexate and other folate analogues in vitro to enhance the cytotoxic effects of the antifoalte drugs into said neoplastic or tumor cells in light of the teachings of Garrow and Roy. One of ordinary skill in the art would have been motivated to carry out the above modification *to investigate* whether the exogenous supply of FPGS into tumor cells resistant to methotrexate or folate analogues would restore cytotoxic sensitivity of these cancer cells to methotrexate or folate analogues. (Emphasis added).

Paper No. 10, page 22.

The Examiner also states:

> One of ordinary skill would have a reasonable expectation of success because lowered FPGS activity and decreased polyglutamylation of antifolates are thought to be general mechanisms by which cancer cells become resistant to a wide range of antifolates, and that Roy clearly show that L1210 tumor cells resistant to methotrexate or edatrexate have lowered FPGS activity, coupled with the teachings of Kim demonstrating that FPGS-deficient mutant CHO AUXB1 cells expressing high levels of human FPGS become more sensitive to the cytotoxicity of methotrexate. Therefore, the claimed invention as a whole was prima facie obvious in the absence of evidence to the contrary. (emphasis added).

Paper No. 10, pages 22-23. Applicants respectfully traverse the rejection.

Rejection of claimed subject matter as obvious under 35 U.S.C. § 103 in view of a combination of references requires (1) consideration of whether prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition

or carry out the claimed process, and (2) whether the prior art would also have revealed that such a person would have reasonable expectation of success; both suggestion and reasonable expectation of success must be found in the prior art, not in Applicant's disclosure. *See In re Vaeck*, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991). Further, all claim limitations must be taught or suggested by the prior art. *In re Royka*, 180 USPQ 580 (CCPA 1974).

Applicants assert that, with respect to the newly added claims, the Examiner has not established a *prima facie* case of obviousness because he has not pointed to anything, in the cited references or in the body of knowledge generally possessed by those skilled in the art, that would suggest the modification or combination of the references necessary to arrive at Applicants' claimed invention.

The Examiner states that "One of ordinary skill in the art would have been motivated to carry out the above modification *to investigate* whether the exogenous supply of FPGS into tumor cells resistance to methotrexate or folate analogues would restore cytotoxic sensitivity of these cancer cells to methotrexate or folate analogues." This is a classical "obvious to try" argument, that is not the proper test for obviousness. Obviousness cannot be established absent some teaching, suggestion or incentive, and thus, although it might have been obvious to one skilled in art to try and see if cytotoxic sensitivity of neoplastic cells to anti-folate drugs could be enhanced by introducing an FPGS gene, such evidence does not establish a *prima facie* case of obviousness. See *In re Geiger*, 2 USPQ2d 1276 (Fed. Cir. 1987) and *In re Fine*, 5 USPQ2d 1596 (Fed. Cir. 1988).

In any event, Applicants claims are not directed to restoring cytotoxic sensitivity to neoplastic cells that are resistant to methotrexate or other folate analogues. The present

claims are directed to: "A method of enhancing the cytotoxic sensitivity of neoplastic cells to an antifolate drug, said method comprising:

(a) delivering into said neoplastic cells a vector, said vector comprising a nucleotide molecule encoding folylpolyglutamyl synthetase (FPGS), operably linked to a promoter, *wherein said FPGS is expressed in said neoplastic cells at a level higher than the endogenous FPGS level of said neoplastic cells*;

(b) treating said neoplastic cells with an antifolate drug that is polyglutamated by said FPGS; and

(c) *enhancing the cytotoxic sensitivity of said neoplastic cell to said antifolate drug."* (Emphasis added).

In rationalizing the rejection, the Examiner has relied on the theory in Kim that "[l]owered FPGS activity may be a general mechanism by which cells can become resistant to a wide range of antifolates." This theory, however, does not provide the requisite motivation to modify or combine the cited references, and arrive at the presently claimed invention.

Applicants note that the claims are not dependent on any particular mechanism of action, nor do they necessarily require that the neoplastic cell being treated is MTX resistant. The point of novelty of the currently claimed invention is the teaching and demonstration that, in the context of vector-mediated gene therapy, the elevation of FPGS activity beyond the endogenous level characteristic of a particular tumor cell, will augment their cytotoxic sensitivity.

As discussed above, Kim shows that vector-mediated transfection of FPGS cDNA can restore FPGS activity and reintroduce cytotoxic sensitivity into variant CHO cells that

express NO ENDOGENOUS FPGS activity. In contrast, the claimed invention recites that the FPGS is transferred to neoplastic cells which have some endogenous FPGS activity. Applicants have shown that elevation of FPGS activity via vector-mediated gene therapy, *beyond the endogenous level characteristic of most tumor cells*, will augment their cytotoxic sensitivity. That is, the issue of whether tumor cells, already expressing FPGS, can be imbued with enhanced antifolate sensitivity after FPGS gene delivery has not been previously addressed by any of the cited art, taken alone or in combination.

Applicants contend that the Examiner has not provided a sufficient explanation as to why a person skilled in the art would have been motivated to modify the teachings of Kim or Garrow such that the cloned FPGS gene is delivered, not to a mutant Chinese hamster ovary cell, but to a *neoplastic cell.*

Since there is no motivation to modify or combine the cited references to arrive at Applicants' claimed invention, and the Examiner has failed to point to any such motivation, a *prima facie* case of obviousness has not been established. Accordingly, Applicants respectfully request that the rejection of the claims under 35 USC § 103(a) be reconsidered and withdrawn.

B. The Examiner rejects claims 1, 7-9 and 13 under 35 USC § 103(a), because, according to the Examiner, these claims are unpatentable over Kim *et al., J. Biol. Chem. 268*:21680-21685 (1993) ("Kim") as evidenced by Osborne *et al., J. Biol. Chem. 268*:21657-21664 (1993) ("Osborne"), and in view of Garrow *et al., Proc. Nat'l. Acad. Sci. 89*:9151-9155 (1992) ("Garrow") and Roy *et al., J. Biol. Chem. 272*: 6903-6908 (1997) ("Roy") as applied to claims 1-3, 5-6 and 10-12 above, and further in view of Nakanishi, *Crit. Rev.*

Therapeu. Drug Carrier Systems 12:263-310 (1995)("Nakanishi"). *See* Paper No. 10, pages 23-24. Applicants respectfully traverse the rejection.

The teachings of all references, except Nakansihi, are discussed and distinguished above. Nakanishi reviews various viral vectors, as well as particle bombardment and direct injection of DNA, as a means of gene transfer into cells. The Examiner contends that it would have been obvious for a skilled artisan to use a recombinant retrovirus vector for delivering the FPGS gene into neoplastic cells resistant to methotrexate in the "modified method" resulting from the combined teachings of Kim, Osborne, Garrow and Roy, including means of direct injection into the tumor cells or by particle bombardment. The Examiner contends that one of skill would have been motivated to carry out this modification simply on the designer's choice of vectors.

Nakanishi does not remedy any of the fundamental defects of the prior rejection, see *supra*. Acordingly, this rejection is improper and should be withdrawn.

C. The Examiner rejects claims 1-3 and 5-13 under 35 USC § 103(a), because, according to the Examiner, these claims are unpatentable over Roy *et al.*, *J. Biol. Chem. 272*: 6903-6908 (1997) ("Roy"), in view of Kim *et al.*, *J. Biol. Chem. 268*:21680-21685 (1993) ("Kim"), Garrow *et al.*, *Proc. Nat'l. Acad. Sci. 89*:9151-9155 (1992) ("Garrow") and Roth *et al.*, *J. Nat'l. Cancer Inst. 89*:21-39 (1997) ("Roth"). Applicants respectfully traverse this rejection.

This rejection is apparently directed to the *in vivo* aspect of the claimed method.

According to the Examiner, "Roy teaches that *in vitro* L1210 tumor cells resistant to methotrexate have a decrease in the rate of FPGS mRNA transcript formation, resulting

in lower FPGS activity" and that "L1210 tumor cells resistant to edatrxate have constitutively down-regulated steady state levels of FPGS compared with parental L1210 tumor cells." As acknowledged by the Examiner, Roy does not teach to deliver directly into neoplastic cells *in vivo* a vector comprising a nucleotide encoding FPGS, then treating with an antifolate drug to kill the neoplastic cells.

The Examiner then cites Kim for teaching that FPGS-deficient CHO cells expressing FPGS metabolize MTX to polyglutamates characteristic of human cells and that lowered FPGS activity may be a general mechanism by which human leukemia cells become resistant to a wide range of antifolates. The Examiner cites Garrow for the cloning of the human cDNA sequence encoding FPGS and Roth for various cancer gene therapy approaches utilizing viral and non-viral vectors *in vivo*.

Accordingly, the Examiner contends that it would have been obvious to direct delivery of a non-viral or viral vector comprising an FPGS sequenceinto neoplastic cells *in vivo* that may require resistance to MTX and other folate analogues in order to restore their sensitivity to the cytotoxic sensitivity to these drugs. The Examiner again cites Kim as motivation to combine based on Kim's alleged teaching that lowered FPGS activity may be the mechanism by which cancer cells become resistant to a wide range of antifolates and cites Roy as providing a reasonable expectation of success based on his alleged teaching that L1210 tumor cells resistant to methotrexate have lowered FPGS activity.

As discussed above, Kim shows that vector-mediated transfection of FPGS cDNA can restore FPGS activity and reintroduce cytotoxic sensitivity into variant CHO cells that express *no endogenous* FPGS activity. In contrast, the claimed invention recites that the FPGS is transferred to neoplastic cells which have some endogenous FPGS activity.

Applicants have shown that elevation of FPGS activity via vector-mediated gene therapy, *beyond the endogenous level characteristic of most tumor cells*, will augment their cytotoxic sensitivity. That is, the issue of whether tumor cells, already expressing FPGS, can be imbued with enhanced antifolate sensitivity after FPGS gene delivery has not been previously addressed by any of the cited art, taken alone or in combination. The limitations of the claimed method have not been met.

Since there is no motivation to modify or combine the cited references to arrive at Applicants' claimed invention, and the Examiner has failed to point to any such motivation, a *prima facie* case of obviousness has not been established. Accordingly, Applicants respectfully request that the rejection of the claims under 35 USC § 103(a) be reconsidered and withdrawn.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Version with markings to show changes made

Claims 1-13 have been canceled.

Claims 14-32 have been added.